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Characterization of Fibrous assembly from Lime Peel Extract

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ABSTRACT. The present research focuses on the development of anti-oxidant and antimicrobial polymeric fibrous material based on lime peel extract which contains many bioactive compounds having medicinal and healthcare properties. The aim of the present research is to generate fibrous assembly from lime peel extract and characterize the newly developed fibrous assembly in terms of chemical group identification, burning behavior, solubility behavior, thermal behavior, moisture absorbency and microscopic behavior. The antibacterial activity is also studied for possible application in the suitable area of medical textiles. The fibrous assembly primarily composed of about lignin, cellulose, hemicelluloses and different polyphenolic compounds such as flavonoid, shows excellent moisture absorbency and antimicrobial property against *E. coli* and *S. aureus* strains. The fibrous assembly has potential for applications in the wound dressing and healthcare sector.

1. INTRODUCTION

Lime (Citrus aurantifolia) is a polyembryonic species with a greenish yellow, smooth surfaced, thin-skin, and a solid core at maturity having highly acidic juice. It has been reported [1, 2] that lime peel extracts have active components like flavonoids and limonene which acts as antioxidant, anti obesity as well as anti carcinogenic agents and also show a tendency to inhibit tumor growth. Until recently, health promoting properties of citrus have always been associated with their content of vitamin C. Only in the last decade studies have focused on several bioactive compounds, specifically limonoids and flavonoids which play a major role in preventing chronic diseases [2]. In addition to vitamin-C, carotenoids, flavonoids, limonoids, phenolic acids when consumed in appropriate quantities are beneficial to human health [3-10]. Growing body of evidence seems to suggest that limonoids and flavonoids have different biological functions, including antioxidative, anti-inflammatory, antibacterial, antiallergic, antiviral, antiproliferative, antimutagenic, and anticarcinogenic activities [11-15].

1.1. Bioactive ingredients in lime peel extracts

Lime peels contain multiple bioactive agents. Consumption of foods rich in flavonoids are known to prevent several degenerative pathologies, including cardiovascular diseases, atherosclerosis, cataract and several forms of cancer [16]. Among the flavonoids, hesperidin was found to be the most abundant in lime peels [17]. Apart from antioxidant activity, hesperidin is also known to act as anticancer agent through prostaglandin and inhibitor of chemical carcinogens[18]. The other major flavonoid reported in peel is rutin, which is also known as quercetin-3-rutinoside. Rutin has shown significant scavenging properties on oxidizing species, such as hydroxyl radical, superoxide radical and peroxyl radical. Furthermore, it has shown antiallergic, anti-inflammatory, antitumor, antibacterial, antiviral and anti-protozoal properties [19].

1.2. Antioxidant activities of bioflavonoid

Antioxidant activity of lime fractions was attributed to its hydrogen donating ability [20, 21], may be, due to the presence of flavonoids, carotenoids and ascorbic acid [22]. The flavonoid can scavenge oxygen species in two ways i.e. Hydrogen Atom Transfer (HAT) and Electron transfer (ET). The

mechanisms of antioxidant action can include inhibition of reactive oxygen species formation by suppressing enzymes involved in free radical production; scavenging reactive oxygen species; and protecting antioxidant defenses [23]. Flavonoids inhibit the enzymes responsible for superoxide anion production, such as xanthine oxidase and protein kinase [24]. Besides scavenging, flavonoids may stabilize free radicals involved in oxidative processes by complexing with them [25].

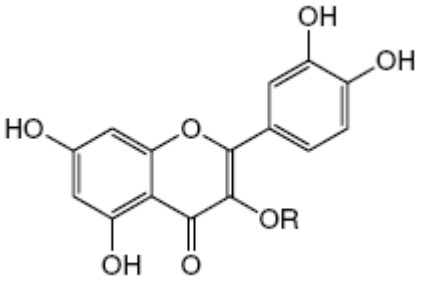

Fig. 1. Structure of flavonoid

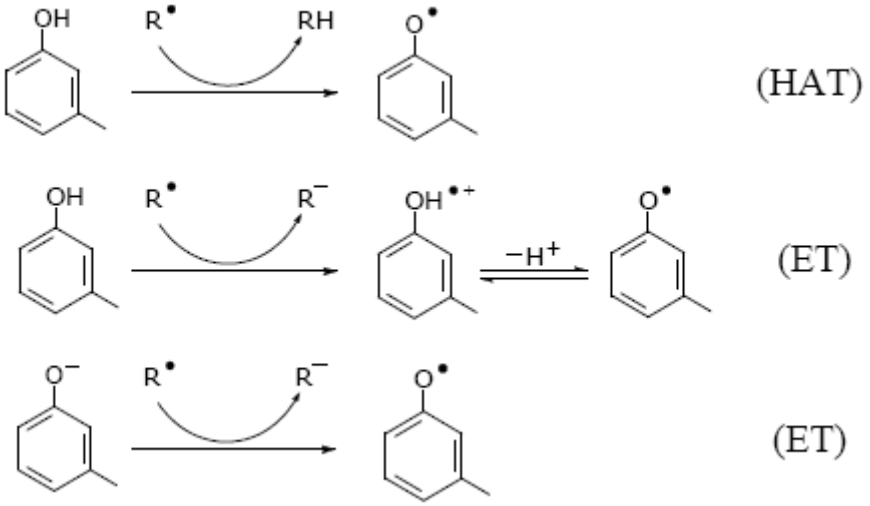

Fig. 2. Reaction mechanism for antioxidant activity

The antioxidant capacity of flavonoids is directly related to their structure (Figure 1 & 2) [26], and in the case of hesperidin, the presence of a hydroxyl group at position 3 of ring B is responsible for the capacity of hesperidin to scavenge the hydroxyl radicals generated from hydrogen peroxide. It is already known that the ability to scavenge superoxide is due to a hydroxyl group at position C-4 of ring B [26. 27].

All the above studies relates to possibilities of using lime peel extracts in various health care related applications. There is no work reported so far in the direction of development of fibrous assembly from lime peel extracts and its application in the form of fibrous assembly. Again there is no reported literature about the behavior and properties of this type of fibrous assembly. So in this research paper, an attempt has been made to develop the fibrous assembly from lime peel extract and characterized the newly developed fibrous assembly in terms of microscopy, chemical group identification, elemental analysis, X-ray, thermal behavior, moisture absorbency, solubility and antibacterial activity. This approach is of interest due to probable wound care and tissue engineering applications where polymers having antimicrobial property show several advantages.

2. MATERIALS AND EXPERIMENTAL PROCEDURE

The materials used for development of fibrous assembly are lime peel. The lime peels are extracted from fresh limes procured from local market of jalandhar. Then lime peel extracts i.e. liquid from lime peel is collected by cold pressing of fresh lime peel. The collected lime peel extracts are

subsequently spread on a rubbery surface. The fibrous assemblies are produced on an aluminum foil by pressing the liquid with a plastic surface in such a manner that the web of fibrous assembly are formed and collected on the aluminum foil when the plastic surface is removed from the liquid. This manual mechanical process is repeated till the required amount of fibrous assembly is formed. The fibrous assembly was kept in desiccator for drying in presence of P_20_5 at 25^0C until constant mass was reached. A film is prepared by dissolving the same fibrous assembly in methylene chloride solvent using solution casting method.

2.1. Microscopic and EDX analysis of fibrous assembly

The newly developed fibrous assembly is analyzed by Leica optical microscope, Scanning Electron Microscope (SEM) and Energy Dispersive X-ray Spectroscopy (EDX). For optical microscopy, the measurement was taken at 100 different places of the fibrous assembly at 2.5x magnification. Finally the average diameter of the fibrous assembly was measured. The samples were examined under SEM using Zeiss EVO 18 Special edition. Examination was done with working distance of 6.5 mm and Electron Gun frequency at 20 kV for imaging at a magnification of 10KX. The x-ray energy dispersive spectrometer by OXFORD (OXFORD INCA) was used to analyze the elemental compositions analysis of the fibrous assembly.

2.2. Burning behavior of fibrous assembly

A small bunch of fibrous assembly is tested for burning behavior as per standard ASTM D 4391. In this test method the fibrous assembly is twisted and introduced towards flame and then into the flame to allow burning and finally removed from the flame.

2.3. Solubility behavior of the fibrous assembly

The newly developed fibrous assembly was characterized in terms of solubility using Stratmann Series according to ASTM standard D 276.

2.4. Moisture absorbency behavior

The weight of freshly extracted fibrous assembly was measured by Sartorius micro balance at room temperature and 65±2% relative humidity. Then the fibrous assembly was tested using standard method ASTM-2495(2001) for measuring moisture regain and moisture content percentage. In this method the fibrous assembly was dried in a desiccator in presence of phosphorus pentoxide. After 72 hours, the weight of the fibrous assembly was again measured. The dried material was placed inside another desiccator in presence of Calcium Chloride atmosphere to maintain 65% relative humidity. Then the weight of the fibrous assembly was taken after the equilibrium is achieved. The experiments were repeated for 50 different samples.

2.5. FTIR Spectroscopy (Fourier Transformation Infrared Spectroscopy)

Perkin Elmer (Spectrum BX) FTIR was used for identification of chemical group in the fibrous assembly. A total of 100 scans per sample were taken with a resolution of 4 cm^{-1} in the frequency range of 400 cm^{-1} to 4000 cm^{-1} in the transmission mode using KBr window.

2.6. Thermal analysis

The TGA, DTA and DTG curves were recorded on an EXSTAR6000 (TG/DTA 6300) instrument at a heating rate of 10^0C/min from 25^0C to 700^0C. The DSC curves were recorded on METTLER TOLEDO (DSC 823^e) instrument at a heating rate of 1^0, 5^0, and 10^0 C/min from 25^0 C to 150^0C.

2.7. Antibacterial activity

The antibacterial effect was investigated using the shake flask test in accordance with GB 15979-2002 Hygienic Standard for disposable sanitary products[28]. The test bacteria were Staphylococcus aureus and Escherichia coli. After incubation at 37 ± 1^0c for 36 hours, bacteria were counted. The antibacterial properties of the fibrous assembly were evaluated by calculating the reduction

percentage of bacteria by the formula Xs = (A-B)/ A. Where Xs is the reduction percentage of the bacteria in %, A is the number of bacteria colonies on the agar plate recovered from bacterial solution at 0 contact time. B is the number of bacterial colonies on the agar plate recovered from the specimen after shaking for 1 hour.

3. RESULTS AND DISCUSSION

The freshly extracted fibrous assembly developed from lime peel extract is white in color, giving out a soft hand and pleasant lime fragrance. Initially the fibrous assembly exhibits week mechanical properties in terms of strength. Exact amount of strength is not known.

3.1. Analysis of Morphology of fibrous assembly

Images of the fibrous assembly from Leica microscope and SEM were analyzed to study the morphology of the newly developed fibrous materials.

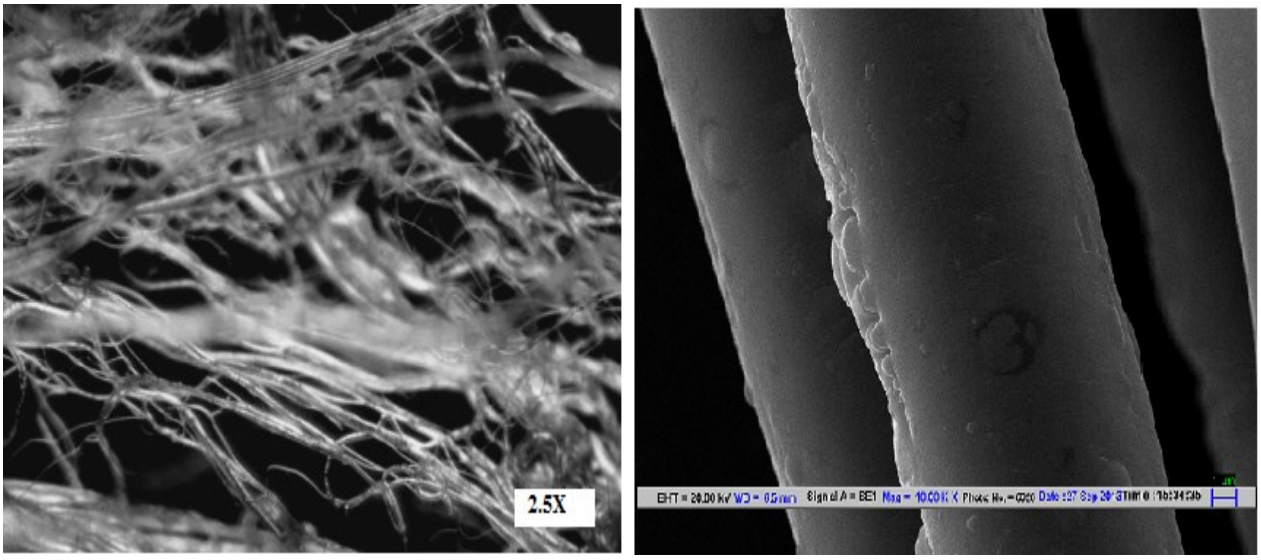

Fig. 3. Microscopic image of the fibrous assembly.

The morphology of fibrous assembly produced from lime peel extracts shows a fibriliar structure with irregular fibre walls. The diameter of the fibre shows variation along the length. The variation in diameter along length may be due to variation of extraction force during manual process of fibre formation. The average diameter of individual fibres is about 8.19 micron (SD 0.87). Spots different shapes have been identified at different places of the body of the fibre. The spots found on the surface of the fibre may be due to the presence of volatile oils in the lime peel extract.

3.2. Burning behavior of fibrous assembly

From the Table 1, it is observed that the fibrous assembly shrinks like synthetic fibre on approaching flame. Burns with a yellow flame like cotton and continues to burn rapidly like rayon with a sweet smell like polyester. A hard round bead is formed like acrylic and acetate.

Table 1. Burning Behavior of Fibrous Assembly.

	Observation
On approaching flame	The fibre shrinks
In the flame	Burn quickly with yellow flame
Out of the flame	Continues to burn rapidly
Residue	Hard round bead is formed
Smell	Sweet chemical smell

3.3. Solubility behavior of fibrous assembly

From Table 2, it has been observed that, the fibrous assembly was dissolved in the solvents like methylene chloride, mixture of heptanes and carbon tetrachloride and cyclohexane at room temperature. At boil, the fibrous assembly was also dissolved in phenol and 40 % sulphuric acid.

Table 2. Solubility Behavior of Fibrous Assembly.

Solvent	Observation
Glacial acetic acid	No change
Acetone	No change
Ethylene diamine (hydrate)	No change
Hydrochloric acid (40%)	No change
Methylene chloride cold	**Fibre dissolved immediately**
Dimethyl formamide	No change
Sulphuric acid (40%)	Partially dissolved and color changes to yellow and fully dissolved at boil
Phenol	Partially dissolved in cold and fully dissolved at boil
Cyclohexane	**Broken into segments and dissolved**
Nitric acid (40%)	Not dissolved but color changes to golden yellow
Formic acid (40%)	No change

3.4. Moisture absorbency behavior

Moisture absorbency behavior was tested for freshly prepared fibrous assembly by taking fifty measurements. The average data has been plotted in figure 4.

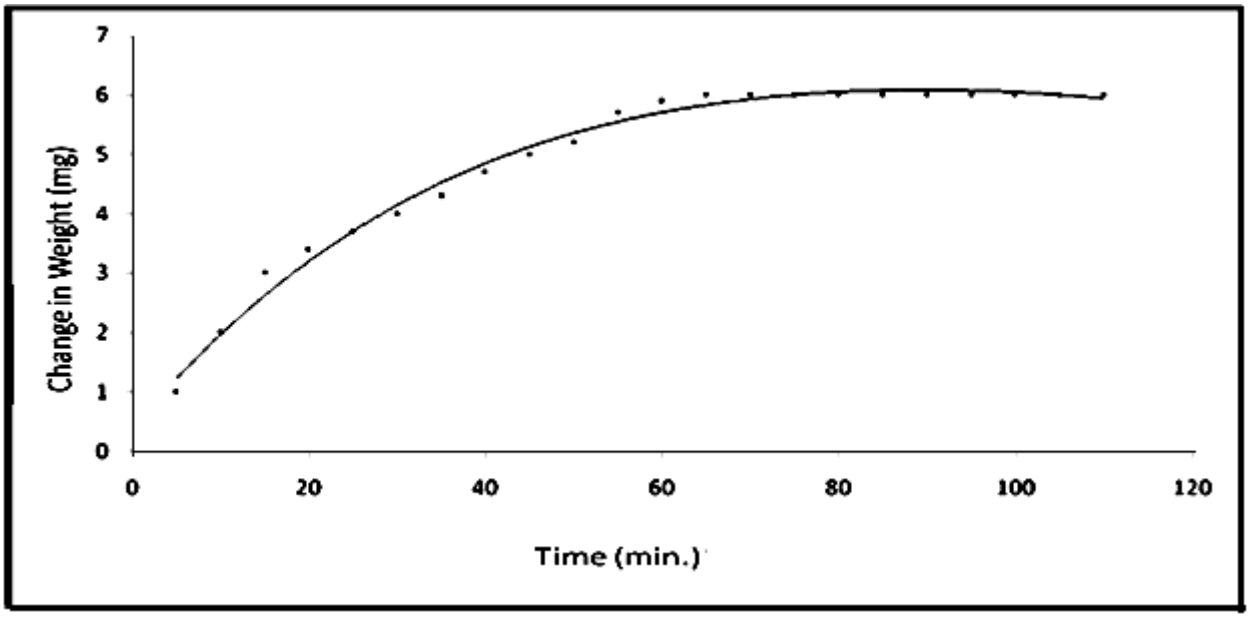

Fig. 4. Moisture absorbency behavior of freshly extracted fibrous assembly.

From figure 4, it has been observed that the freshly extracted fibrous assembly from lime peel extracts absorbs moisture with increase in time and equilibrium achieved after some time. Again the material is dried using chemical like phosphrous pentoxide in desiccators. The average moisture regain and moisture content value was calculated using the following formula

$$\text{Moisture regain} = \frac{weight\ of\ the\ moisture}{dry\ wt.\ of\ sample} \times 100$$

$$\text{Moisture content} = \frac{weight\ of\ the\ moisture}{total\ weight\ of\ the\ sample} \times 100$$

The average moisture regain and moisture content value of the fibrous assembly are 11% and 9 % respectively. This valuable property of the fibrous assembly is also upheld by their high moisture retention capability may be due to the amorphous structure of the fibre assembly.

3.5. Analysis of elemental composition

The EDX spectra of the fibrous assembly are shown in Figure 5. All the results are calculated in weight %.

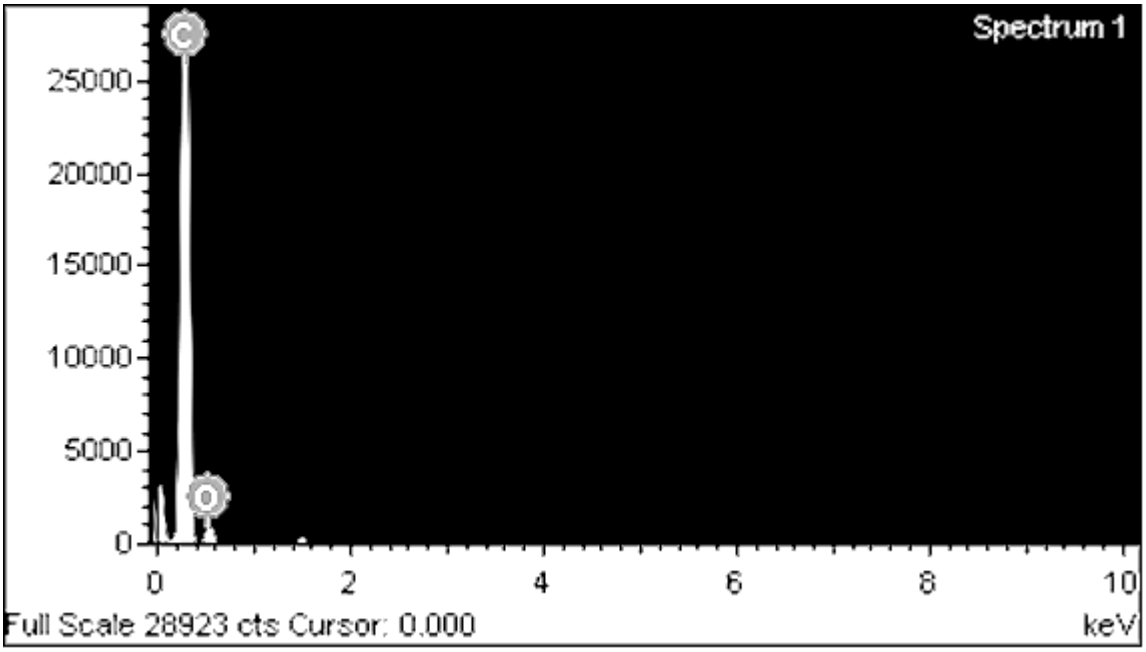

Fig. 5. EDX spectra of fibrous assembly.

The EDX analysis of fibrous assembly identified two major elements present in the fibrous assembly i.e. carbon and oxygen. The weight percentage of carbon and oxygen found in the fibrous assembly was approximately 91 and 8 respectively. .

3.6. FTIR Spectroscopy

The FTIR spectra of newly developed fibrous assembly were recorded in figure 6. The most intense band at 3322 cm^{-1} was assigned to the stretching of –OH groups of the carbohydrates and those of lignin[29]. The signals at 2913 and 2851 cm^{-1} are caused by asymmetrical and symmetrical stretching vibrations of C–H groups. The band at 1732 cm^{-1} was assigned to the carbonyl (C=O) stretching. The intense band at 1030 cm^{-1} corresponds to the link C–O–R while the distinctive band around 1265 cm^{-1} was attributed to aliphatic chains (–CH_2– and –CH_3) forming the basic structure of this lignocelluloses materials[30]. Most of the bands have contributions from both carbohydrates (cellulose and hemicellulose) and lignin.

It follows from analysis of literature [31, 32] on IR spectra of fibrous assembly containing flavonoids that the carbonyl group bands have the following vibration frequencies like 1603cm^{-1} and 1631 cm^{-1} are the characteristics peak of fisetin and hesperitin type of flavonoid respectively.

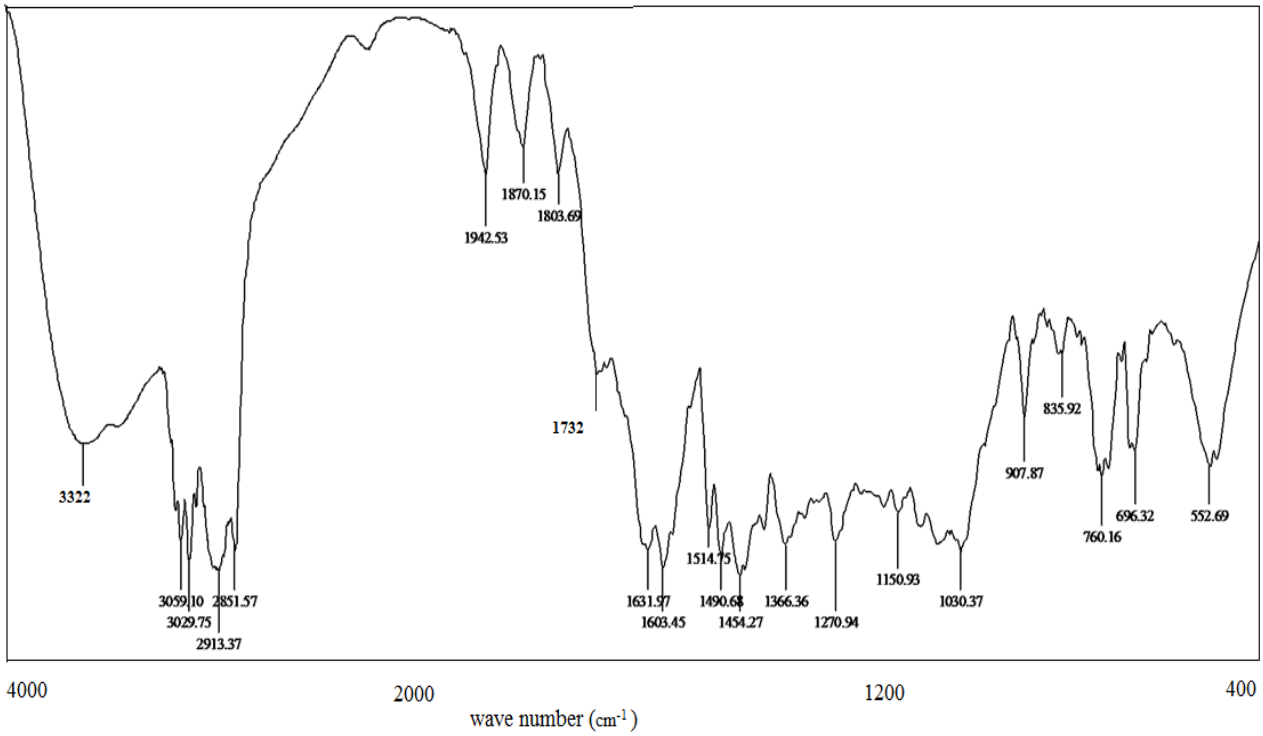

Fig. 6. Spectroscopy of fibrous assembly.

Briggs and Colebrook [31] analysed the shifts of valance vibrations bands of the flavonoid carbonyl group according to the number of OH groups and their position in the benzene rings. The position and number of hydroxyl groups in the flavonoid rings seem to have a great influence here. The –C-H bending is available at wave number 1270 cm^{-1} for querecetin type of flavonoid. Other typical absorption bands characteristics of pure cellulose are that at wave number 1631, 1031, 907, 3322 cm^{-1} [33].

3.7. Thermal analysis by DSC (Differential Scanning Calorimetry)

The DSC curves of fibrous assembly at different rate of heating are shown in Figure 7.

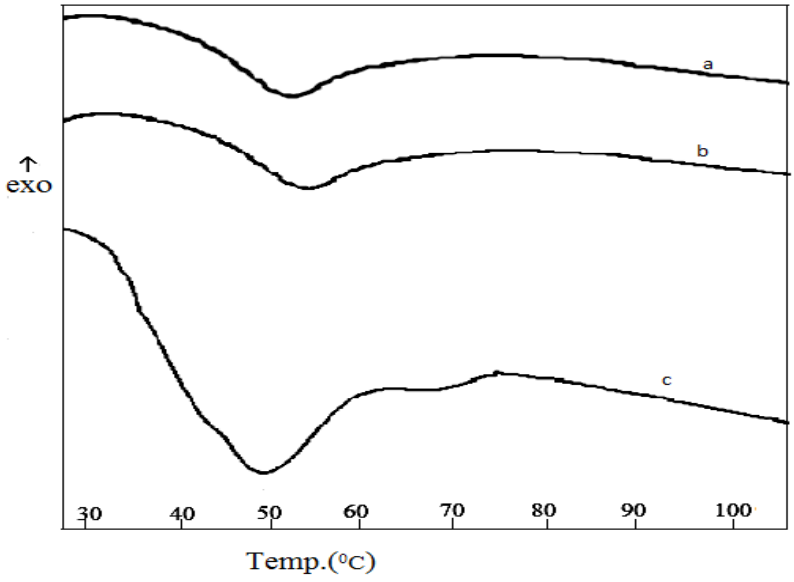

Fig. 7. DSC curves of fibrous assembly (a) 1 °C/min, (b) 5 °C/min and (c) 10°C/min

The endothermic peak at 1°C/min, 5°C/min and 10°C/min rate of heating were observed may be due to the presence of various volatile oils and moisture in the fibrous assembly. In all the cases the

endothermic peak is observed at a temperature range of 50 to 60^{0}C. The fibrous assembly is predominantly amorphous which indicates less number of crystals present in the fibrous assembly. So there is no significant melting peak is shown in DSC curve.

3.8. TGA Analysis

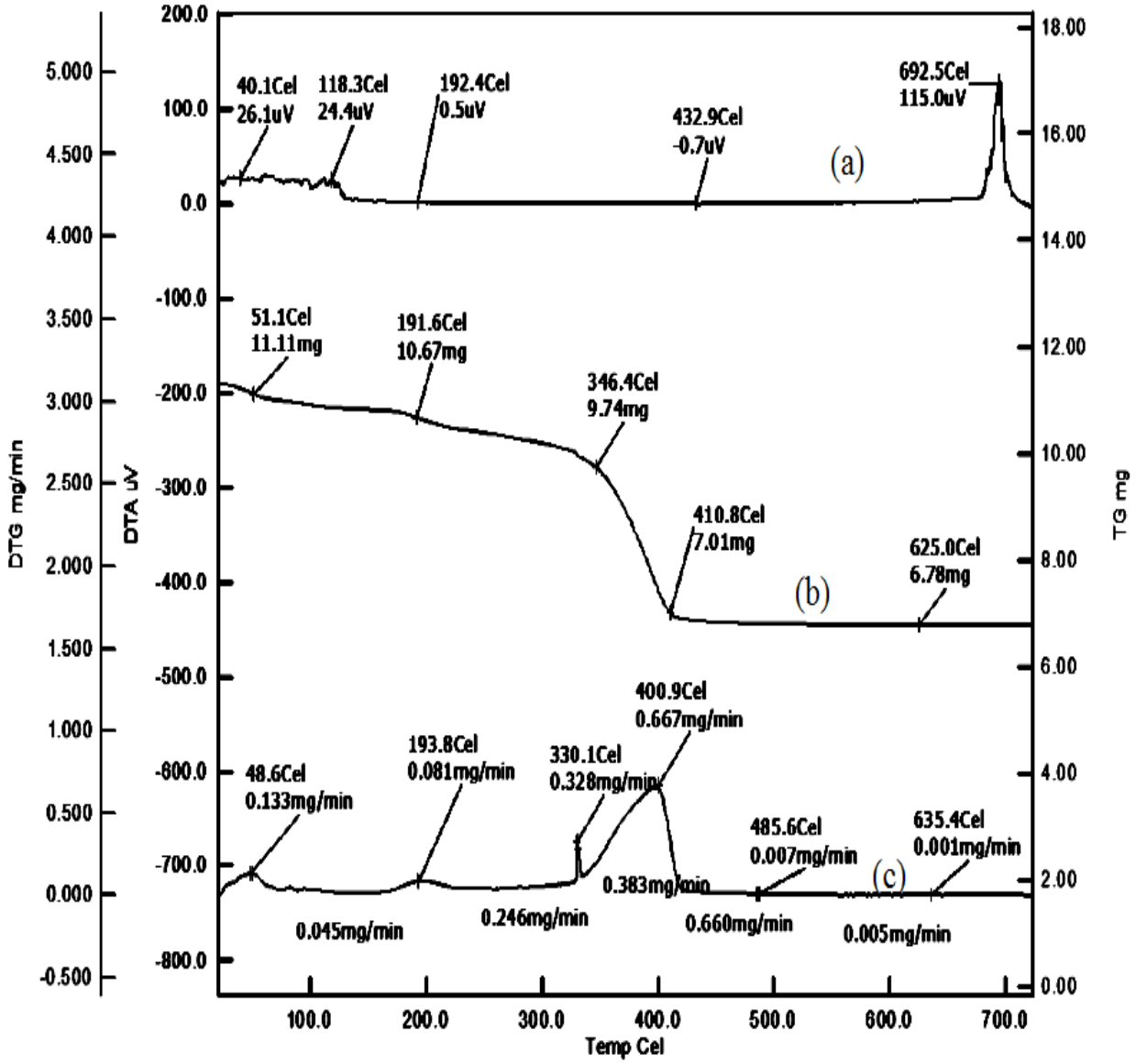

Fig. 8 a. DTA curve, **b.** TGA curve and **c.** DTG curve

Corresponding to the weight loss on TG curves at least three main thermal events can be clearly distinguished up to 625 °C. In all TGA curves[36] (Figure 8), the first weight loss about 4% below 190 °C refers to volatile components and physically adsorbed water molecules in the samples. The main mass losses are associated to the biomass decomposition, essentially, to its three main components (hemicellulose, cellulose and lignin). The second step weight loss about 8% from 190 to 350 °C can be attributed to decomposition of hemicelluloses. The third decomposition process starts with a weight loss of 28% between 350 and 410 °C was associated to the degradation of cellulose and the carbon-carbon linkage between lignin structural units was cleaved in this temperature. Above 410^{0}C, the process becomes stable with a weight loss about 39%.

3.9. Antibacterial behavior

Antibacterial effect of 100% lime peel extracts fibrous assembly with different bacterial species in terms of percentage reduction of bacteria is shown in Table 3.

Table 3. (Antibacterial activity of newly developed fibrous assembly)

Sl. no	Sample thickness in mm	E. Coli (percentage reduction of bacteria) (mean value of three replicates)	S. aureus (percentage reduction of bacteria) (mean value of three replicates)
1	1	86.25 ± 2 SD	98.71 ± 2 SD
2	1.5	98.00 ± 2.5 SD	99.21 ± 2.5 SD
3	2	97.00 ± 2.4 SD	98.00 ± 2.6 SD
4	2.5	97.22 ± 2 SD	96.25 ± 2.1 SD
5	3	87.53 ± 2.3 SD	89.00 ± 2.2 SD

All the samples were very effective against both test bacteria with a reduction of over 86 % for E. Coli and 98 % for S. aureus indicating excellent antibacterial property. The reason behind the antibacterial property may be due to presence of different flavonoids and other polyphenolic compounds present in the fibrous assembly as per literature.

4. CONCLUSIONS

In this research work, the fibrous assembly was successfully produced from the organic biopolymer extracted from lime peel. The fibrous assembly produced from lime peel extract composed of more than 90 % of carbon and oxygen. The color of the fibrous assembly is white. The fibrous assembly exhibit fibriliar morphology with irregular surface as shown in SEM image. The fibrous assembly has also been characterized in terms of burning and solubility behavior. Five solvents are identified for dissolution of fibrous assembly i.e. methylene chloride, cyclohexane, phenol at boil, 40% sulphuric acid at boil and a mixture of carbon tetrachloride and heptanes. The average moisture regain and moisture content value of the freshly extracted fibrous assembly were 11% and 9 % respectively. The presence of different types of bioactive flavonoids like quercetin, hesperitin and cellulose, hemicelluloses and carbohydrates and lignin etc. in the inherent structure of fibrous assembly has been identified by FTIR spectroscopy. The fibrous assembly behaves like thermoplastic during heating as is evident from DSC. It is clear from TGA result, the weight loss of the fibrous assembly starts at a temperature around 51^0C. About 4 % of weight loss has been indetified between 50-190^0C. from 190-350^0C, the weight loss is about 8%. Then the fibrous assembly starts degradation from 350^0C as per TGA curve. Above 410^0C, the process becomes stable as it is observed both from TGA and DTG curve. The antibacterial activities of the fibrous assembly were found excellent against E. Coli and S. aureus. So from the above results it is concluded that, the fibrous materials may play an important role in the process of wound healing and tissue regeneration.

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